

(b) at least one additional component;
wherein said composition does not comprise a biological extract of animal or cellular origin, or a living nourishing substrate.--

--20. The composition of claim 19, wherein said at least one additional component comprises at least one member selected from the group consisting of a non-ionic water-soluble polymer and an oil-plus-surfactant mixture.--

--21. The composition of claim 19, wherein the components of the complex nutrient medium are biocompatible, biomimetic, and bioavailable with respect to the skin.--

--22. The composition of claim 19, wherein the complex nutrient medium has the following composition, the concentration of the components being expressed in milligrams per liter of solvent:

Amino acids

| | |
|------------------------------------|--------|
| L-Alanine | 9.2 |
| L-Arginine HCl | 421.4 |
| L-Asparagine (anhydrous) | 14.2 |
| L-Aspartic acid | 4.0 |
| L-Cysteine HCl · H ₂ O | 42.0 |
| L-Glutamic acid | 14.8 |
| L-Glutamine | 1754.4 |
| Glycine | 7.6 |
| L-Histidine HCl · H ₂ O | 50.0 |
| L-Isoleucine | 6.0 |
| L-Leucine | 131.2 |
| L-Lysine HCl | 54.0 |
| L-Methionine | 13.5 |

| | |
|-----------------------------------|-------|
| L-Phenylalanine | 10.0 |
| L-Proline | 34.6 |
| L-Serine | 126.1 |
| L-Threonine | 24.0 |
| L-Tryptophan | 9.3 |
| L-Tyrosine 2 Na 2H ₂ O | 11.7 |
| L-Valine | 70.3 |

Vitamins and cell growth factors

| | |
|-------------------------|------|
| d-Biotin | 0.02 |
| Folic acid | 0.80 |
| Nicotinamide | 0.04 |
| Ca D-Pantothenate | 0.30 |
| Pyridoxine HCl | 0.06 |
| Riboflavin | 0.04 |
| Thiamine HCl | 0.30 |
| Vitamin B ₁₂ | 0.41 |
| i-Inositol | 18.0 |
| Putrescine 2 HCl | 0.20 |
| Sodium pyruvate | 55.0 |
| Thymidine | 0.73 |
| Adenine (HCl) | 24.0 |
| DL-Lipoic acid | 0.20 |

Inorganic components

| | |
|---------------------------------------|--------|
| Sodium chloride | 6800.0 |
| KCl | 112.0 |
| Na ₂ HPO ₄ | 284.0 |
| CuSO ₄ · 5H ₂ O | 0.003 |

| | |
|---|--------------------|
| Sodium acetate | 300.0 (anhydrous) |
| D-Glucose | 1080.0 |
| HEPES (piperazine) | 6600.0 |
| Phosphorylethanolamine | 0.06768 |
| Ethanolamine | 0.04684 |
| Sodium sulphate | 3.4 |
| Sodium bicarbonate | 1160.0 |
| FeSO ₄ · 7H ₂ O | 1.39 |
| MgCl ₂ · 6H ₂ O | 120.0 |
| CaCl ₂ · 2H ₂ O | from 13.0 to 22.05 |
| ZnSO ₄ · 7H ₂ O | 0.144 |
| (NH ₄) ₆ MO ₇ O ₂₄ · 4H ₂ O | 0.00120 |
| Na ₂ SiO ₃ · 5H ₂ O | 0.142 |
| MnCl ₂ · 4H ₂ O | 0.00002 |
| SnCl ₂ · 2H ₂ O | 0.00011 |
| NH ₄ VO ₃ | 0.00057-- |

--23. The composition of claim 19, wherein the complex nutrient medium comprises a phase that is biocompatible with the superficial parts of the human body and wherein the complex nutrient medium is distributed homogeneously within said phase.--

--24. The composition of claim 19, wherein the composition is a medicinal composition.--

--25. A pharmaceutical formulation base comprising the composition of claim 24.--

--26. The pharmaceutical formulation base of claim 25, wherein said base is used for preservative treatment of grafts.--

--27. The pharmaceutical formulation base of claim 25, wherein said base is used for prevention or treatment of disorders and/or delay of cicatrization.--

--28. The composition of claim 20, comprising two phases, wherein a first phase comprises an aqueous continuous phase containing the complex nutrient medium.--

--29. The composition of claim 28, wherein the composition is a medicinal composition.--

--30. A pharmaceutical formulation base comprising the composition of claim 29.--

--31. The composition of claim 20, comprising two phases, wherein a first phase comprises an oily continuous phase and a second phase comprises a discontinuous phase containing said complex nutrient medium.--

--32. The composition of claim 31, wherein the composition is a medicinal composition.--

--33. A pharmaceutical formulation base comprising the composition of claim 32.--

--34. A cosmetic base comprising the composition of claim 19.--

--35. A cosmetic preparation comprising the cosmetic base of claim 34, wherein said complex nutrient medium constitutes either an active principal or an excipient.--

--36. The cosmetic preparation of claim 35, wherein said excipient potentiates an active principal.--

--37. A method of manufacturing a composition for topical use, said method comprising combining:

(a) a complex nutrient medium comprising at least some amino acids, a vitamin, a cell growth factor, and an inorganic salt, wherein said medium supports viable *in vitro* growth of human epidermal keratinocytes with at least one clonal proliferation of said keratinocytes in the first passage, and

(b) at least one additional component,
wherein said composition resulting from said method does
not comprise a biological extract of animal or cellular origin,
or a living nourishing substrate.--

--38. The method of claim 37, wherein said at least one
additional component comprises at least one member selected from
the group consisting of a non-ionic water-soluble polymer and an
oil-plus-surfactant mixture.--

--39. The method of claim 37, wherein said complex
nutrient medium has the following composition, the concentration
of the components being expressed in milligrams per liter of
solvent:

A
Amino acids

| | |
|------------------------------------|--------|
| L-Alanine | 9.2 |
| L-Arginine HCl | 421.4 |
| L-Asparagine (anhydrous) | 14.2 |
| L-Aspartic acid | 4.0 |
| L-Cysteine HCl · H ₂ O | 42.0 |
| L-Glutamic acid | 14.8 |
| L-Glutamine | 1754.4 |
| Glycine | 7.6 |
| L-Histidine HCl · H ₂ O | 50.0 |
| L-Isoleucine | 6.0 |
| L-Leucine | 131.2 |
| L-Lysine HCl | 54.0 |
| L-Methionine | 13.5 |
| L-Phenylalanine | 10.0 |
| L-Proline | 34.6 |
| L-Serine | 126.1 |

| | |
|-----------------------------------|------|
| L-Threonine | 24.0 |
| L-Tryptophan | 9.3 |
| L-Tyrosine 2 Na 2H ₂ O | 11.7 |
| L-Valine | 70.3 |

Vitamins and cell growth factors

| | |
|-------------------------|------|
| d-Biotin | 0.02 |
| Folic acid | 0.80 |
| Nicotinamide | 0.04 |
| Ca D-Pantothenate | 0.30 |
| Pyridoxine HCl | 0.06 |
| Riboflavin | 0.04 |
| Thiamine HCl | 0.30 |
| Vitamin B ₁₂ | 0.41 |
| i-Inositol | 18.0 |
| Putrescine 2 HCl | 0.20 |
| Sodium pyruvate | 55.0 |
| Thymidine | 0.73 |
| Adenine (HCl) | 24.0 |
| DL-Lipoic acid | 0.20 |

Inorganic components

| | |
|---------------------------------------|-------------------|
| Sodium chloride | 6800.0 |
| KCl | 112.0 |
| Na ₂ HPO ₄ | 284.0 |
| CuSO ₄ · 5H ₂ O | 0.003 |
| Sodium acetate | 300.0 (anhydrous) |
| D-Glucose | 1080.0 |
| HEPES (piperazine) | 6600.0 |

| | |
|---|--------------------|
| Phosphorylethanolamine | 0.06768 |
| Ethanolamine | 0.04684 |
| Sodium sulphate | 3.4 |
| Sodium bicarbonate | 1160.0 |
| $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ | 1.39 |
| $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ | 120.0 |
| $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ | from 13.0 to 22.05 |
| $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ | 0.144 |
| $(\text{NH}_4)_6\text{MO}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ | 0.00120 |
| $\text{Na}_2\text{SiO}_3 \cdot 5\text{H}_2\text{O}$ | 0.142 |
| $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ | 0.00002 |
| $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ | 0.00011 |
| $\text{NH}_4 \text{VO}_3$ | 0.00057-- |

--40. A method of medicinal or cosmetic treatment

comprising contacting a human or animal with a composition comprising at least some amino acids, a vitamin, a cell growth factor, and an inorganic salt, wherein said composition does not comprise a biological extract of animal or cellular origin, or a living nourishing substrate, and wherein said composition supports viable *in vitro* growth of human epidermal keratinocytes with at least one clonal proliferation of said keratinocytes in the first passage.--

--41. The method of claim 40, wherein the composition is biocompatible, biomimetic, and bioavailable with respect to skin.--

--42. The method of claim 40, wherein said composition comprises the following components, the concentration of the components being expressed in milligrams per liter of solvent:

Amino acids

| | |
|------------------------------------|--------|
| L-Alanine | 9.2 |
| L-Arginine HCl | 421.4 |
| L-Asparagine (anhydrous) | 14.2 |
| L-Aspartic acid | 4.0 |
| L-Cysteine HCl · H ₂ O | 42.0 |
| L-Glutamic acid | 14.8 |
| L-Glutamine | 1754.4 |
| Glycine | 7.6 |
| L-Histidine HCl · H ₂ O | 50.0 |
| L-Isoleucine | 6.0 |
| L-Leucine | 131.2 |
| L-Lysine HCl | 54.0 |
| L-Methionine | 13.5 |
| L-Phenylalanine | 10.0 |
| L-Proline | 34.6 |
| L-Serine | 126.1 |
| L-Threonine | 24.0 |
| L-Tryptophan | 9.3 |
| L-Tyrosine 2 Na 2H ₂ O | 11.7 |
| L-Valine | 70.3 |

Vitamins and cell growth factors

| | |
|-------------------|------|
| d-Biotin | 0.02 |
| Folic acid | 0.80 |
| Nicotinamide | 0.04 |
| Ca D-Pantothenate | 0.30 |
| Pyridoxine HCl | 0.06 |
| Riboflavin | 0.04 |

| | |
|-------------------------|------|
| Thiamine HCl | 0.30 |
| Vitamin B ₁₂ | 0.41 |
| i-Inositol | 18.0 |
| Putrescine 2 HCl | 0.20 |
| Sodium pyruvate | 55.0 |
| Thymidine | 0.73 |
| Adenine (HCl) | 24.0 |
| DL-Lipoic acid | 0.20 |

Inorganic components

| | |
|---|--------------------|
| Sodium chloride | 6800.0 |
| KCl | 112.0 |
| Na ₂ HPO ₄ | 284.0 |
| CuSO ₄ · 5H ₂ O | 0.003 |
| Sodium acetate | 300.0 (anhydrous) |
| D-Glucose | 1080.0 |
| HEPES (piperazine) | 6600.0 |
| Phosphorylethanolamine | 0.06768 |
| Ethanolamine | 0.04684 |
| Sodium sulphate | 3.4 |
| Sodium bicarbonate | 1160.0 |
| FeSO ₄ · 7H ₂ O | 1.39 |
| MgCl ₂ · 6H ₂ O | 120.0 |
| CaCl ₂ · 2H ₂ O | from 13.0 to 22.05 |
| ZnSO ₄ · 7H ₂ O | 0.144 |
| (NH ₄) ₆ MO ₇ O ₂₄ · 4H ₂ O | 0.00120 |
| Na ₂ SiO ₃ · 5H ₂ O | 0.142 |
| MnCl ₂ · 4H ₂ O | 0.00002 |
| SnCl ₂ · 2H ₂ O | 0.00011 |

$\text{NH}_4 \text{ VO}_3$

0.00057.--

--43. A method of culturing keratinocytes *in vitro* comprising incubating human epidermal keratinocytes in the presence of a complex nutrient medium comprising at least some amino acids, a vitamin, a cell growth factor, and an inorganic salt, wherein said complex nutrient medium does not comprise a biological extract of animal or cellular origin, or a living nourishing substrate, and wherein said medium supports viable *in vitro* growth of human epidermal keratinocytes with at least one clonal proliferation in the first passage.--

--44. A composition for topical use comprising:

(a) a complex nutrient medium that permits viable *in vitro* growth of human epidermal keratinocytes with at least one clonal proliferation of said keratinocytes in the first passage, and

(b) at least one additional component,

wherein said composition does not comprise any biological extract of animal or cellular origin or a living, nourishing substrate.--

--45. The composition of claim 44, wherein said at least one additional component comprises at least one member selected from the group consisting of a non-ionic water-soluble polymer and an oil-plus-surfactant mixture.--

--46. The composition of claim 44, wherein the components of the complex nutrient medium are biocompatible, biomimetic, and bioavailable with respect to the skin.--

--47. The composition of claim 44, wherein the complex nutrient medium comprises a phase that is biocompatible with the

superficial parts of the human body and wherein the complex nutrient medium is distributed homogeneously within said phase.--

--48. The composition of claim 44, wherein the composition is a medicinal composition.--

--49. A pharmaceutical formulation base comprising the composition of claim 48.--

--50. The pharmaceutical formulation base of claim 49, wherein said base is used for preservative treatment of grafts.--

--51. The pharmaceutical formulation base of claim 49, wherein said base is used for prevention or treatment of disorders and/or delay of cicatrization.--

--52. The composition of claim 45, comprising two phases, wherein a first phase comprises an aqueous continuous phase containing the complex nutrient medium.--

--53. The composition of claim 52, wherein the composition is a medicinal composition.--

--54. The composition of claim 45, comprising two phases, wherein a first phase comprises an oily continuous phase and a second phase comprises a discontinuous phase containing said complex nutrient medium.--

--55. The composition of claim 54, wherein the composition is a medicinal composition.--

--56. A pharmaceutical formulation base comprising the composition of claim 55.--

--57. The composition of claim 54, wherein said first phase is in emulsion form.--

--58. A cosmetic base comprising the composition of claim 45.--

--59. A cosmetic preparation comprising the cosmetic base of claim 58, wherein said complex nutrient medium constitutes either an active principal or an excipient.--

--60. The cosmetic preparation of claim 59, wherein said excipient potentiates an active principal.--

--61. A method of manufacturing a composition for topical use, said method comprising combining:

(a) a complex nutrient medium that permits viable *in vitro* growth of human epidermal keratinocytes with at least one clonal proliferation of said keratinocytes in the first passage, and

(b) at least one additional component,

wherein said composition does not comprise any biological extract of animal or cellular origin or a living, nourishing substrate.--

--62. The method of claim 61, wherein said at least one additional component comprises at least one member selected from the group consisting of a non-ionic water-soluble polymer and an oil-plus-surfactant mixture.--

--63. The method of claim 61, wherein the components of said complex nutrient medium are biocompatible, biomimetic, and bioavailable with respect to the skin.--

--64. The method of claim 61, wherein the complex nutrient medium comprises a phase that is biocompatible with the superficial parts of the human body and wherein the complex nutrient medium is distributed homogeneously within said phase.--

Sub.B2 --65. ~~A method of medicinal or cosmetic treatment comprising contacting a human or animal with a composition that does not comprise either any biological extract of animal or~~

Sub B2

~~cellular origin, or a living nourishing substrate, wherein said composition permits viable *in vitro* growth of human epidermal keratinocytes with at least one clonal proliferation of said keratinocytes in the first passage...~~

--66. The method of claim 65, wherein the composition is biocompatible, biomimetic, and bioavailable with respect to skin.--

--67. The method of claim 65, wherein the method is used for the preservative treatment of grafts.--

*A1
CONC*

--68. The method of claim 65, wherein the method is used for preventing or treating disorders and/or delay of cicatrization.--

--69. A method of culturing keratinocytes *in vitro* comprising incubating human epidermal keratinocytes in the presence of a complex nutrient medium that does not comprise either any biological extract of animal or cellular origin, or a living nourishing substrate, wherein said complex nutrient medium permits viable *in vitro* growth of human epidermal keratinocytes with at least one clonal proliferation of said keratinocytes in the first passage...--

REMARKS

Claims 19-69 are pending. Claims 1-18 are cancelled herein. It was previously agreed that the claims originally labelled 20[sic] and 21[sic] would be disregarded. Thus, the present Amendment is drafted as if such claims never existed.

This Preliminary Amendment has been made in order to place the claims in better condition for examination by the U.S.